

REVIEW

Besifloxacin: Efficacy and Safety in Treatment and Prevention of Ocular Bacterial Infections

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Received: February 8, 2016 / Published online: March 24, 2016
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ABSTRACT

This comprehensive review summarizes the mechanism of action, pharmacokinetics, efficacy, and safety of besifloxacin ophthalmic suspension, 0.6% and examines its role in the treatment of ocular surface bacterial infections. Besifloxacin possesses balanced activity against bacterial topoisomerase II (also called DNA gyrase) and topoisomerase IV. It has shown a low potential to select for bacterial resistance in vitro and demonstrated strong in vitro activity against many Gram-positive, Gram-negative, and anaerobic organisms, including methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis* (MRSA and MRSE, respectively). Ocular pharmacokinetic studies have shown that besifloxacin achieves high, sustained concentrations in the tear fluid and

conjunctiva following topical administration, with negligible systemic exposure. Large randomized, controlled clinical trials have established the efficacy and safety of besifloxacin administered three times daily for 5 days for treatment of acute bacterial conjunctivitis in both adults and children, with high rates of clinical resolution (up to more than 70% by day 5) and bacterial eradication (more than 90% by day 5), and a low incidence of adverse events. Additionally, besifloxacin applied twice daily for 3 days demonstrated greater efficacy than vehicle in treating bacterial conjunctivitis. Case reports, a large retrospective chart review, and animal studies have provided supporting evidence for the efficacy of besifloxacin in the management of acute bacterial keratitis. There is some evidence to suggest that besifloxacin may provide an advantage over other current-generation fluoroquinolones in antimicrobial prophylaxis for ocular surgery. Besifloxacin is an appropriate option for treatment of bacterial conjunctivitis, and its use in the treatment of bacterial keratitis and lid disorders, as well as for surgical prophylaxis, appears promising and warrants further evaluation.

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Keywords: Acute bacterial conjunctivitis; Antibiotic resistance; Bacterial keratitis infections; Besifloxacin; MRSA; MRSE; Surgical antibiotic prophylaxis; Topical ophthalmic fluoroquinolones

INTRODUCTION

Fluoroquinolones (FQs) have been successfully used in ophthalmology for nearly two decades, thanks in large part to a series of incremental improvements in their antimicrobial activity and pharmacokinetic profiles [1]. Today, treatment for bacterial ocular surface infections—including conjunctivitis, blepharitis, and keratitis—is largely empirical; the FQs' broad-spectrum antimicrobial activity and documented safety and lack of toxicity make them well suited to empirical therapy [2–4]. With activity against a broad spectrum of bacterial pathogens including Gram-positive, Gram-negative and anaerobic organisms [1, 5], current-generation FQs, such as gatifloxacin, moxifloxacin, and besifloxacin, have become first-line agents for the treatment and prevention of bacterial ocular infections [6, 7].

As with other antibiotics, resistance to FQs has developed [2, 8]. Until the early 2000s, FQ resistance was uncommon among ocular pathogens, but with the rapid increase in clinical utilization of FQs (both systemic and topical), resistance has begun to emerge [8]. Surveillance studies have shown an alarming trend of increasing resistance in ocular isolates over the past two decades [9–14]. Most notably, pathogenic strains such as methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant *Staphylococcus epidermidis* (MRSE) are becoming prevalent, and many strains show multidrug resistance including resistance to both earlier and current generation FQs.

The most recent addition to the topical ocular FQ family is besifloxacin, an FQ developed solely for topical ophthalmic use. A 0.6% besifloxacin ophthalmic suspension (Besivance[®], Bausch + Lomb, Rochester, NY, USA) was approved in the US and Canada for the treatment of bacterial conjunctivitis in 2009 [15, 16]. The first topical chlorofluoroquinolone, besifloxacin has a unique molecular structure designed to confer increased antibacterial potency [4, 17]. In susceptibility assays, besifloxacin demonstrated potent in vitro activity against a wide range of pathogens, including those that are resistant to other FQs and antibacterial classes [5].

Perhaps the most distinctive feature of besifloxacin is its lack of a systemic formulation. Unlike all other ophthalmic FQs, besifloxacin has never been used systemically, nor has it been used in agriculture or animal husbandry [2]. Because extensive systemic antibiotic use and antibiotic use in agriculture are two major drivers of resistance development among bacteria [18–20], it has been suggested that the limitation to ocular use may slow the development of bacterial resistance to besifloxacin, although cross-resistance from other FQs is still possible [8].

This article reviews our current knowledge of besifloxacin, looking at mechanisms of action, pharmacokinetic properties, in vitro antimicrobial activity, and, most importantly, clinical efficacy and safety. The goal is to review and evaluate besifloxacin's current and potential roles in treatment and prevention of ocular surface bacterial diseases.

METHODS

A literature search was conducted in October 2015 of the Medline[®], Biosis Previews[®], and Embase[®] databases, employing “besifloxacin” or

“Besivance” as the search terms. The search was limited to English-language articles, and 156 papers/abstracts were retrieved. Primary articles, review articles, and abstracts on the mechanism of action, pharmacokinetic properties, or clinical efficacy and safety of besifloxacin were identified ($n = 52$), and additional relevant articles were collected from the references of selected publications. This article is based on previously conducted studies, and does not involve any new studies of human or animal subjects performed by any of the authors.

RESULTS AND DISCUSSION

Mechanism of Action

FQs act by inhibiting two enzymes essential for bacterial DNA synthesis: topoisomerase II (DNA gyrase) and topoisomerase IV [21, 22]. Topoisomerase II relaxes supercoils of double-stranded bacterial DNA to facilitate DNA replication; topoisomerase IV is responsible for unlinking daughter chromosomes to allow their segregation into two daughter cells at the end of each round of replication, an action known as decatenation. Binding with these DNA-tethered enzymes to form FQ–enzyme–DNA complexes, the FQs exert their effect by inhibiting the topoisomerases, blocking DNA replication, and, ultimately, killing the bacterial cell [6, 22].

Older FQs, such as ofloxacin and ciprofloxacin, preferentially bind one of the essential enzymes [6]. In most Gram-negative bacteria, topoisomerase II tends to be the primary target; in Gram-positive organisms, the primary target is typically topoisomerase IV [6, 23]. However, newer agents, such as gatifloxacin and moxifloxacin, are believed to possess dual activity, with more comparable targeting activity against both topoisomerases

[6, 24]. This dual-binding mechanism of action increases antimicrobial activity, particularly activity against Gram-positive pathogens [6]. Additionally, potent inhibition of both topoisomerases is thought to reduce spontaneous emergence of resistance, as two point mutations are needed in the enzymes to confer resistance to the FQ, and double mutations, as a genetic event, rarely occur [25, 26].

Besifloxacin, the newest ophthalmic FQ, likewise targets both enzymes but has been shown to act through potent, balanced inhibition of both topoisomerase II and topoisomerases IV [27]. Results of enzymatic activity assays by Cambau et al. [27] suggested that, in *S. pneumoniae*, besifloxacin has greater in vitro activity against topoisomerase II and IV than moxifloxacin or ciprofloxacin. The 50% inhibitory concentration (IC_{50}) of besifloxacin against *S. pneumoniae* topoisomerase II was four- to eightfold lower than that of moxifloxacin and 15-fold lower than that of ciprofloxacin. Against *S. pneumoniae* topoisomerase IV, the concentration of besifloxacin required to inhibit 50% of isolates was two- and fivefold lower than moxifloxacin and ciprofloxacin, respectively. Consistent with the idea that balanced dual activity against the essential bacterial enzymes reduces the emergence of bacterial resistance, besifloxacin-resistant *S. aureus* and *S. pneumoniae* mutants emerged in vitro at rates nearly two orders of magnitude lower than those of mutants resistant to ciprofloxacin [27], which primarily targets topoisomerase IV in Gram-positive bacteria [6, 27].

Beyond its antimicrobial activity, besifloxacin might provide anti-inflammatory efficacy in ocular infections [28, 29]. Zhang et al. observed inhibitory effects on synthesis of pro-inflammatory cytokines with besifloxacin

in ocular and nonocular cells exposed to stimulants such as interleukin-1 β and lipopolysaccharide [28, 29]. This ability to attenuate inflammatory cytokine responses in stimulated cells has also been found in other FQs [30].

Antibacterial Activity

Progress from one FQ generation to the next was primarily driven by structural modification to the quinolone backbone in an effort to produce broadened and enhanced bactericidal activity, primarily activity against Gram-positive aerobes and anaerobic organisms [1, 23, 31, 32] (Fig. 1). Nalidixic acid, the first quinolone antibiotic and the one from which FQs were subsequently derived, has very low activity against aerobic Gram-positive organisms. Early FQ compounds first achieved added activity against Gram-positive staphylococci from the addition of a fluorine at C-6 (hence the name FQs) and a cyclic

diamine piperazine at C-7 of the quinolone nucleus. Later, the addition of a cyclopropyl side chain at position N-1, as in ciprofloxacin, yielded a wider spectrum of activity against both Gram-positive and Gram-negative aerobes. The current generation FQs, including gatifloxacin and moxifloxacin, have a methoxy substituent at the C-8 position and, consequently, additional activity against Gram-positive and anaerobic bacteria [6, 33].

The besifloxacin molecule (Fig. 2) has a chemical structure similar to gatifloxacin and moxifloxacin but differs in that it has a chlorine atom at the C-8 position (replacing the C-8 methoxy group in moxifloxacin and gatifloxacin) and an amino-azepinyl group at the C-7 position (replacing the pyrrolol-pyridinyl and methyl-piperazinyl substituents in moxifloxacin and gatifloxacin, respectively) [4]. It is this unique combination of 8-chloro and 7-azepinyl substituents, rather than either moiety alone, that is believed to be responsible for besifloxacin's improved

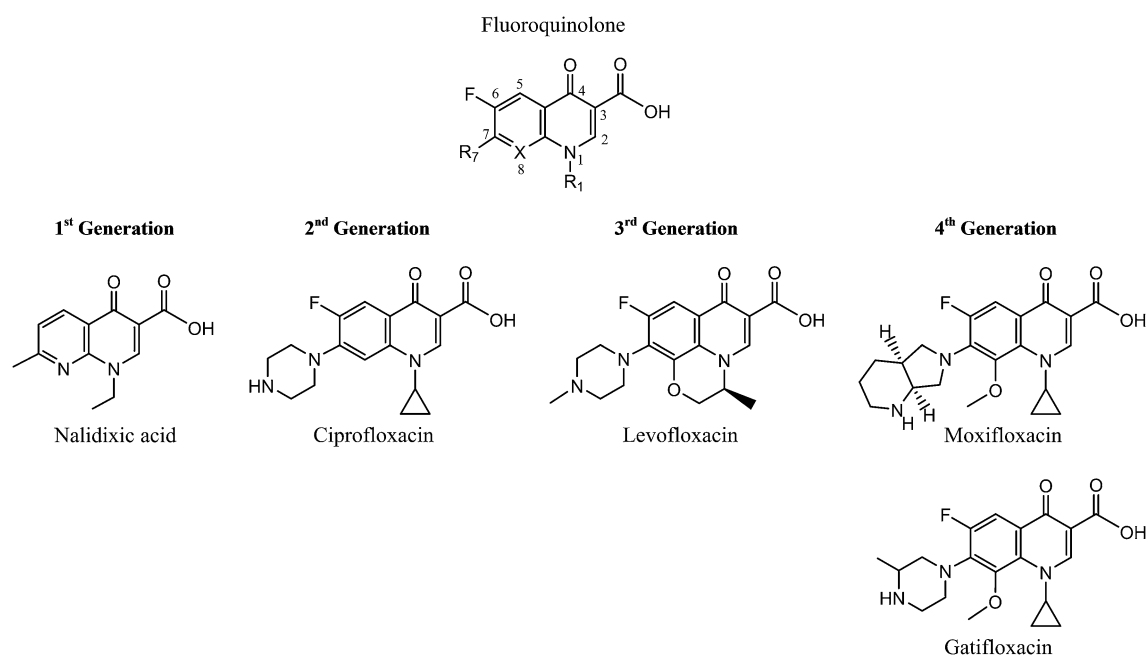


Fig. 1 Structural evolution of fluoroquinolones

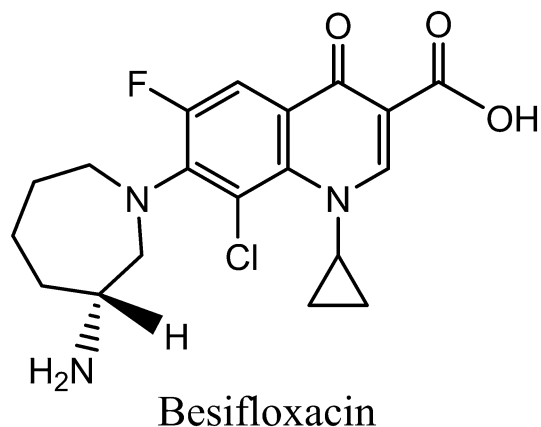


Fig. 2 Chemical structure of besifloxacin (7-[(3R)-3-aminohexahydro-1H-azepin-1-yl]-8-chloro-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid)

antibacterial potency compared with other current generation FQs [4].

Haas et al. [5] evaluated besifloxacin's in vitro activity using 2690 bacterial clinical isolates of 40 species, collected in the US from ocular and respiratory specimens between 2005 and 2008. Consistent with its relatively balanced dual targeting of topoisomerase II and topoisomerase IV [27], minimum inhibitory concentration (MIC) values showed besifloxacin to be active against a broad spectrum of Gram-positive, Gram-negative, and anaerobic organisms, including those commonly associated with ocular infections: *S. aureus*, *S. epidermidis*, *S. pneumoniae*, and *Haemophilus influenzae* [5]. Among various topical agents commonly used for the treatment of ocular infections—including moxifloxacin, gatifloxacin, azithromycin, and tobramycin—besifloxacin had the best in vitro activity (lowest MICs) against Gram-positive pathogens and anaerobes and equivalent or better activity against most Gram-negative isolates [5]. In particular, besifloxacin displayed better activity against resistant strains, including MRSA and ciprofloxacin-nonsusceptible staphylococci,

and activity equal to or better than that of other tested FQs against ciprofloxacin-nonsusceptible *Pseudomonas aeruginosa* isolates.

The activity profile of besifloxacin was confirmed by Haas et al. [34] in an integrated analysis of microbial data from three besifloxacin clinical trials. Across the three trials, a total of 1324 bacterial conjunctivitis isolates, representing more than 70 species, were obtained from patients in the US and Asia. Results from in vitro MIC testing showed that, overall, besifloxacin (0.06 $\mu\text{g}/\text{mL}$ for MIC₅₀ and 0.25 $\mu\text{g}/\text{mL}$ for MIC₉₀, against all isolates) was more potent than comparator FQs (0.125–0.5 $\mu\text{g}/\text{mL}$ for MIC₅₀ and 0.5–2 $\mu\text{g}/\text{mL}$ for MIC₉₀, against all isolates). While the besifloxacin MIC₉₀ against Gram-negative organisms was 0.5 $\mu\text{g}/\text{mL}$ compared to 0.125 and 0.25 $\mu\text{g}/\text{mL}$ for the other FQs tested, besifloxacin was the most potent agent tested against Gram-positive pathogens, including organisms resistant to other FQs. Against ciprofloxacin-resistant MRSA and MRSE, besifloxacin had 16- to 128-fold and 8- to 64-fold greater activity, respectively, in comparison with other tested FQs.

Miller et al. [35] observed similar results in comparing the in vitro efficacy of besifloxacin and comparator antibiotics against 243 ocular staphylococcal isolates collected from a variety of ocular surface and intraocular infections between 2003 and 2008. Besifloxacin was the most potent FQ tested, with an MIC₉₀ (4 $\mu\text{g}/\text{mL}$) eightfold lower than that for moxifloxacin and 16-fold lower than that for ciprofloxacin. Of note, besifloxacin maintained a relatively high potency against staphylococci that were both ciprofloxacin and methicillin resistant, with an MIC₉₀ of 4 $\mu\text{g}/\text{mL}$ for ciprofloxacin-resistant MRSA and 8 $\mu\text{g}/\text{mL}$ for ciprofloxacin-resistant MRSE.

Complementing these in vitro efficacy findings, Haas et al. [36, 37] demonstrated besifloxacin's rapid bactericidal effect on four major ocular pathogens—*S. aureus*, *S. epidermidis*, *S. pneumoniae*, and *H. influenzae*—using minimum bactericidal concentration (MBC)/MIC and time-kill assays. The MBC/MIC ratios of besifloxacin were ≤ 4 for 97.5% of all isolates tested ($n = 120$) [36]. For the Gram-positive pathogens, in particular, besifloxacin had the lowest MIC₉₀ (0.06–4 mg/L) and MBC₉₀ (0.12–4 mg/L) of all agents tested, which included not just FQs but also select beta-lactams, macrolides, and aminoglycosides. Regardless of the resistance phenotype of the isolates, besifloxacin was bactericidal within 45–120 min, at least 2- to 4-times faster than, and at lower concentrations than, gatifloxacin or moxifloxacin [37].

It is noteworthy that all of the previously mentioned besifloxacin studies documented a high potency and bactericidal activity against resistant organisms. This is further supported by the five-year results of the Antibiotic Resistance Monitoring in Ocular Microorganisms (ARMOR) study, a nationwide bacterial resistance ocular surveillance study initiated in 2009 to determine the susceptibility and resistance profiles of *S. aureus*, coagulase-negative staphylococci (CoNS), *S. pneumoniae*, *H. influenzae*, and *P. aeruginosa* isolates from ocular infections [14]. From 2009 through 2013, more than 3000 isolates were contributed by 72 eye care centers, community hospitals, and academic or university hospitals across the US. The MIC results for these isolates, particularly the staphylococci, showed a significant level of antibiotic resistance: methicillin/oxacillin-resistant strains accounted for 42.2% and 49.7% of *S. aureus* and CoNS isolates, respectively, and multidrug resistance (defined as ≥ 3 antibiotic classes) was

prevalent among these methicillin-resistant strains (86.8% for MRSA and 77.3% for methicillin-resistant CoNS). Among the FQs tested, besifloxacin demonstrated the greatest in vitro potency against staphylococcal isolates, especially the methicillin-resistant strains. Its MIC₉₀ against MRSA (2 $\mu\text{g}/\text{mL}$) and methicillin-resistant CoNS (4 $\mu\text{g}/\text{mL}$) were comparable to those of vancomycin (1 and 2 $\mu\text{g}/\text{mL}$, respectively).

Bacterial resistance to FQs arises primarily from mutations in bacterial topoisomerase II and topoisomerase IV genes [38, 39]. That besifloxacin demonstrated greater potency against FQ-resistant strains suggests that besifloxacin is less affected by topoisomerase mutations compared with older FQs. A previous study demonstrated that, as the number of mutations in genes encoding DNA gyrase and topoisomerase IV increases, MIC values increase for all tested FQs (besifloxacin, moxifloxacin, gatifloxacin, ciprofloxacin, and levofloxacin); the magnitude of this increase for besifloxacin, however, was the smallest (128-fold between susceptible and the most resistant strains) compared to all other FQs (1024- to 2048-fold) [40]. Drug efflux pumps may contribute to antibiotic resistance in both Gram-positive and Gram-negative organisms [41]. However, this mechanism of resistance does not appear to have a significant effect on susceptibility to newer FQs, including besifloxacin [40, 42].

Pharmacokinetic Properties

Pharmacokinetic properties are important to a drug's in vivo efficacy. In a preclinical study in New Zealand Composite rabbits, Ward et al. [17] observed a concentration gradient in compartments from the tear film to the blood plasma following topical administration of a single dose of besifloxacin ophthalmic

suspension 0.6%. The mean ocular residence times were longer than 7 h. Besifloxacin also demonstrated rapid ocular penetration and sustained retention in Dutch-belted rabbits and two monkey species, with peak concentrations observed within a half hour and measurable levels detected in anterior ocular tissues at all time points through 24 h after a single administration [43, 44]. Proksch et al. [43, 45] assessed ocular pharmacokinetics of besifloxacin in tears of 64 healthy human subjects in a single-center, open-label study (Table 1). Maximum mean besifloxacin concentration in the tear fluid was 610 µg/g at 10 min after a single topical administration. Tear concentrations of 10 µg/g and higher were sustained through 12 h and 1.60 µg/g and higher through 24 h, well above the MIC₉₀ for major ocular pathogens, such as *S. pneumoniae*, *S. aureus*, *S. epidermidis*, and *H. influenzae* [5]. The elimination half-life of besifloxacin in human tears was estimated to be 3.4 h.

The commercially available besifloxacin 0.6% ophthalmic suspension is formulated with a mucoadhesive polymer (DuraSite[®], InSite Vision Inc., Alameda, CA, USA) that enhances

Table 1 Pharmacokinetic parameters for besifloxacin 0.6% in human tears after a single instillation

Parameter	Value
C _{max} , mean ± SD	610 ± 540 µg/mL
T _{max}	10 min
AUC _{0–24h}	1232 µg h/g
Elimination half-life	3.4 h
Systemic C _{max} *	<0.5 ng/mL

AUC_{0–24h} area under the curve during 24 h, C_{max} maximum concentration observed, SD standard deviation, T_{max} time at which maximum concentration is observed

* Measured in patients with bacterial conjunctivitis following three times daily dosing [43]

drug retention on the ocular surface [46]. Extended contact time implies that high drug concentrations are retained at the site of infection; for concentration-dependent antibiotics such as FQs, higher drug concentrations mean greater microbial eradication rates. Proksch et al. [47] determined and compared the ocular pharmacokinetics of besifloxacin, moxifloxacin, and gatifloxacin in rabbits using the commercial formulations of each drug. When compared to moxifloxacin and gatifloxacin, a single instillation of besifloxacin (50 µL) resulted in noticeably higher concentrations and prolonged retention of besifloxacin on the ocular surface, with concentrations of 90 µg/g or higher sustained in tears through 8 h after dosing. With regard to area under the curve during 24 h (AUC_{0–24})/MIC₉₀ ratio, a parameter widely used to predict the clinical efficacy of FQs, a single dose of besifloxacin achieved values of about 800 in tears when tested against ciprofloxacin-resistant MRSE and ciprofloxacin-resistant MRSA, far exceeding the target level of ≥30–50 for effective killing of Gram-positive bacteria [47]. By comparison, the AUC_{0–24}/MIC₉₀ ratios were below 10 for both moxifloxacin and gatifloxacin.

In a comparative study evaluating conjunctival drug concentrations of besifloxacin, moxifloxacin, and gatifloxacin after use of commercial topical FQ formulations in humans, besifloxacin achieved the greatest AUC_{0–24}/MIC₉₀ ratio for resistant and non-resistant staphylococcal strains [48]. Results from this single-center, randomized, double-masked, active-controlled study of 108 healthy volunteers demonstrated that conjunctival concentrations of besifloxacin exceeded the MIC₉₀ against of methicillin-susceptible *S. aureus* (MSSA) and *S. epidermidis* (MSSE) for at least 2 h after a single instillation. Mean residence time in the

conjunctiva for besifloxacin was 4.7 h, the longest among the FQs assessed (3.0 h for moxifloxacin; 2.9 h for gatifloxacin).

Several studies have investigated anterior chamber penetration of besifloxacin and other FQs after topical ocular application. In contrast to high ocular surface concentrations, topical administration of FQs generally results in low intraocular levels. A parallel, double-masked clinical trial by Yoshida et al. [49] randomized 50 cataract surgery patients to receive besifloxacin or moxifloxacin. Thirty minutes after repeated preoperative instillation (4 drops, once every 10 min), the mean aqueous concentration of besifloxacin was 0.03 µg/mL, while that of moxifloxacin was 1.61 µg/mL. Thus, when compared with the MIC₉₀ values for *S. epidermidis* and *S. aureus*, neither of the FQs achieved meaningful aqueous humor levels [50]. Donnenfeld et al. [51] assessed aqueous humor concentrations of besifloxacin, moxifloxacin, and gatifloxacin 1 h following topical instillation of a single drop of each drug in 105 patients undergoing uncomplicated cataract surgery. Mean aqueous humor concentrations for besifloxacin, moxifloxacin, and gatifloxacin were 0.13, 0.67, and 0.13 µg/mL, respectively, well below the MIC₉₀ of these agents against methicillin-resistant and ciprofloxacin-resistant *S. epidermidis* and *S. aureus* isolates. Evans et al. compared the aqueous penetration of besifloxacin and moxifloxacin applied 4 times daily for 3 days and 1 drop at 6, 4, 2, and 1 h prior to surgery in a randomized, parallel-group study of 120 cataract surgery patients [52]. Mean penetration levels for besifloxacin and moxifloxacin, respectively, were 0.049 and 0.489 µg/mL at 1 h and 0.047 and 0.329 µg/mL at 6 h.

Chung et al. [53] compared the intraocular penetration of FQs after topical instillation into

rabbit eyes. The mean maximum concentrations of besifloxacin, moxifloxacin, gatifloxacin, and levofloxacin following single instillation were reported to be 1.13, 10.15, 3.33, and 10.67 µg/g in the cornea, 3.70, 1.56, 0.95, and 1.99 µg/g in the bulbar conjunctiva, and 0.11, 1.86, 0.64, and 2.24 µg/g in the aqueous humor, respectively [53]. At 1 h following repeated instillation (4 times, every 15 min), the FQs' respective peak concentrations were 1.91, 13.69, 6.99, and 22.60 µg/g in the cornea, 2.09, 1.48, 0.78, and 4.51 µg/g in the bulbar conjunctiva, and 0.19, 2.47, 1.29, and 5.52 µg/g in the aqueous humor. Repeated instillation (4 times at 15-min intervals) of besifloxacin resulted in an average of 1.3 times higher concentrations of besifloxacin in ocular tissues (bulbar conjunctiva, cornea, aqueous humor, and anterior vitreous), the smallest increase among all FQs assessed (2.1 times on average) [53]. The authors attributed this "surface-retentive" nature of besifloxacin to its DuraSite vehicle and concluded that besifloxacin has favorable pharmacokinetic properties for the treatment of bacterial conjunctivitis and superficial bacterial keratitis.

Efficacy Profile

Besifloxacin ophthalmic suspension 0.6% is currently indicated for the treatment of bacterial conjunctivitis caused by susceptible organisms [15]. It has also been evaluated for the treatment of bacterial keratitis, prophylaxis of postsurgical infections, and treatment of lid disorders but is not currently indicated for those uses.

Bacterial Conjunctivitis

Acute bacterial conjunctivitis is normally a self-limiting condition, but topical antibiotic

therapy can speed clinical and microbiological resolution, reduce the severity of the infection, and lower the risk of complications such as keratitis [54, 55].

The clinical and microbiological efficacy of besifloxacin administered 3 times daily for 5 days for the treatment of bacterial conjunctivitis was established in three randomized, double-masked, controlled clinical trials. Two compared besifloxacin with its vehicle [56, 57], and one compared besifloxacin with moxifloxacin [58]. A fourth randomized, vehicle-controlled clinical study evaluated the efficacy of besifloxacin 0.6% administered twice daily for 3 days in the treatment of bacterial conjunctivitis [59]. In all four trials, clinical resolution (defined as the absence of conjunctival discharge and bulbar conjunctival injection) and bacterial eradication (defined as absence of ocular bacterial species present or above threshold at baseline) were measured at similar time points (day 5 ± 1 or day 8/9). The results are summarized in Table 2.

Karpecki et al. [56] compared besifloxacin with its vehicle in a multicenter phase II study of 269 patients with acute bacterial conjunctivitis, of which 118 were culture-confirmed. The besifloxacin group demonstrated better clinical and microbiological outcomes at day 8 or 9 ($P < 0.001$) compared with the vehicle group (Table 2). The most commonly isolated bacterial species in this study were *H. influenzae* (31.7%), *S. pneumoniae* (27.6%), *S. aureus* (13.8%), and *S. epidermidis* (4.8%). Besifloxacin showed high rates of eradication of each of these species at day 4 ± 1, in agreement with its low MIC₉₀ (0.06–0.25 µg/mL) against these pathogens. Tepedino et al. [57] reported similar efficacy results from a vehicle-controlled phase III study that enrolled 957 patients with acute bacterial

Table 2 Clinical and microbiological outcomes in besifloxacin clinical trials

References	Comparator	Primary assessment	Clinical resolution, % (n/N)		Bacterial eradication, % (n/N)	
			Besifloxacin	Comparator	Besifloxacin	Comparator
Karpecki et al. [56] *	Vehicle	Visit 3 (day 8 or 9)	73.3 (44/60) ^a	43.1 (25/58) ^a	88.3 (53/60) ^a	60.3 (35/58) ^a
Tepedino et al. [57] *	Vehicle	Visit 2 (day 5 ± 1)	45.2 (90/199) ^b	33.0 (63/191) ^b	91.5 (182/199) ^c	59.7 (114/191) ^c
McDonald et al. [58] *	Moxifloxacin	Visit 2 (day 5 ± 1)	58.3 (147/252) ^d	59.4 (167/281) ^d	93.3 (235/252) ^c	91.1 (256/281) ^c
DeLeon et al. [59]**	Vehicle	Visit 2 (day 4 or 5)	65.9 (89/135) ^a	44 (62/141) ^a	85.2 (115/135) ^a	54.6 (77/141) ^a

* Treatment regimen: three times daily dosing, 5 days

** Treatment regimen: twice daily dosing, 3 days

^a $P < 0.001$

^b $P = 0.0084$

^c $P < 0.0001$

^d 95% confidence interval for non-inferiority, -9.48 to 7.29; $P > 0.05$

^e 95% confidence interval for non-inferiority, -2.44 to 6.74; $P > 0.05$

conjunctivitis at 58 sites in the US. In the 390 patients with culture-confirmed acute bacterial conjunctivitis, besifloxacin achieved significantly better clinical and microbiological outcomes at both analysis time points (day 5 ± 1; day 8 or 9) compared with vehicle (Table 2).

A third multicenter phase III clinical trial was conducted in the US and Asia to evaluate and compare the clinical and antimicrobial efficacy of besifloxacin 0.6% and moxifloxacin 0.5% (Vigamox®; Alcon, Fort Worth, TX, USA) [58, 60]. The study randomized 1161 patients, of whom 533 had culture-confirmed acute bacterial conjunctivitis. The two antibiotic treatments produced comparable clinical and microbiological outcomes on both days five and eight ($P > 0.05$; Table 2). In the group of patients with *S. aureus* infection, the rate of clinical resolution on day 8 was significantly higher with besifloxacin treatment compared with moxifloxacin (84.5% vs. 70.2%, $P = 0.0291$).

A post hoc analysis of data from 815 pediatric patients (447 with culture-confirmed bacterial conjunctivitis) who had participated in the three clinical trials confirmed that besifloxacin maintained its clinical and antimicrobial efficacy in children and adolescents (1–17 years of age) [61]. In a multicenter, randomized study of 33 neonatal patients (≤ 31 days of age) with presumed bacterial conjunctivitis, besifloxacin 0.6% and gatifloxacin 0.3% given three times daily for seven days demonstrated similar efficacy, with high rates of clinical resolution (over 70%) and microbial eradication (about 90%) for both groups on day 8 or 9 [62].

DeLeon et al. [59] evaluated besifloxacin administered twice daily for 3 days compared with vehicle in 474 patients with bacterial conjunctivitis (276 were culture-confirmed). At

day 4 or 5, both clinical and microbiological outcomes were significantly better ($P < 0.001$) in the besifloxacin group than in the vehicle group (Table 2). At day 7 ± 1, 4 days after treatment discontinuation, rates of bacterial eradication remained greater in the besifloxacin group ($P < 0.001$), whereas rates of clinical resolution were not different ($P = 0.209$).

In addition to its efficacy against the common bacterial pathogens involved in conjunctivitis, besifloxacin 0.6% has demonstrated efficacy against less frequently encountered species. *P. aeruginosa*, a Gram-negative bacterium notorious for its ability to cause severe keratitis, and which accounts for about 5% of bacterial conjunctivitis cases and up to one-third of bacterial keratitis infections [12, 63]. Turaka et al. [64] described a single case of giant fornix syndrome associated chronic conjunctivitis that was caused by *P. aeruginosa* and successfully treated with besifloxacin. Silverstein et al. [65] performed a post hoc analysis of clinical outcomes in patients with bacterial conjunctivitis due to *P. aeruginosa* across all four aforementioned besifloxacin clinical studies. Of 1317 patients with culture-confirmed bacterial conjunctivitis, 9 patients (0.7%) with *P. aeruginosa* infection were identified, and 5 of these received besifloxacin. Bacterial eradication was achieved in all five patients by the first follow-up visit and clinical resolution was observed in four patients by the second visit. The MIC₉₀ for besifloxacin against all *P. aeruginosa* isolates was 2 µg/mL, well below reported mean concentrations of besifloxacin in human tears following a single topical ocular administration (610 µg/g at 10 min and above 10 µg/g through 12 h) [43]. On the basis of the bacterial eradication and clinical resolution data, a

revision of the original US Food and Drug Administration (FDA)-approved labeling for besifloxacin was made in 2012 to include *P. aeruginosa* as an indicated bacterial pathogen [15].

Using the same integrated data set, the researchers also assessed the clinical efficacy of besifloxacin 0.6% in conjunctivitis cases caused by *Serratia marcescens*, *Neisseria* spp., MRSA, and MRSE [66]. Treatment with besifloxacin resulted in high bacterial eradication rates in all treated infections: 100% by the first follow-up visit for *S. marcescens* ($n = 4$) and *Neisseria* spp. infections ($n = 7$) and 87.8% by the second follow-up visit for infections caused by MRSA ($n = 12$) and MRSE ($n = 37$). The MIC₉₀ for besifloxacin was 1 µg/mL for *S. marcescens*, 0.25 µg/mL for *Neisseria* spp., 0.06 µg/mL for both ciprofloxacin-sensitive MRSA and ciprofloxacin-sensitive MRSE, and 4 µg/mL for both ciprofloxacin-resistant MRSA and ciprofloxacin-resistant MRSE. The proportion of patients with clinical resolution was 100% (7/7) for *Neisseria* spp. infections and 75% (3/4) for *S. marcescens* infections at the second follow-up visit. For staphylococcal infections, the rate of clinical resolution at the first follow-up visit was 1/2 for ciprofloxacin-sensitive MRSA, 2/10 for ciprofloxacin-resistant MRSA, 15/21 for ciprofloxacin-sensitive MRSE, and 6/16 for ciprofloxacin-resistant MRSE; at the second follow-up visit, the respective rates were 1/2, 5/10, 18/21, and 12/16.

Bacterial Keratitis

The clinical efficacy of besifloxacin 0.6% in the treatment of bacterial keratitis has not been evaluated in randomized, controlled studies. Michaud reported a case of a patient with contact lens-related severe keratitis that was successfully treated with a regimen that

included besifloxacin 0.6% [67]. Pandit [68] described a case of a patient with a large corneal ulcer due to *Brevundimonas diminuta*. Following months of treatment with a regimen of multiple topical antibiotic agents, including besifloxacin, the ulcer resolved leaving a minimal corneal scar. A retrospective chart review by Schechter et al. of more than 200 patients with bacterial keratitis found similar treatment outcomes ($P \geq 0.208$) between besifloxacin-treated patients ($n = 142$) and those treated with moxifloxacin ($n = 85$), although the results were confounded by inclusion of other topical antibacterials in the therapeutic regimen for some patients [69]. The frequency and duration of besifloxacin and moxifloxacin use varied but did not differ, with a median duration of 15 days for both treatment groups and a final dosing frequency of 4 or more times daily for the majority of patients. Both treatment groups had high rates of physician-assessed bacterial eradication (95.8% besifloxacin vs. 91.8% moxifloxacin). Evident corneal scarring was noted in 23.2% of besifloxacin-treated patients and 29.4% of moxifloxacin-treated patients and corneal neovascularization in less than 2% of patients in either treatment group.

Animal studies provide further evidence that besifloxacin has great potential as a treatment for bacterial keratitis, especially for infections caused by resistant organisms. In a rabbit model of MRSA keratitis, treatment with besifloxacin 0.6%—early treatment (starting 10 h post-infection) or late treatment (starting 16 h post-infection)—resulted in greater reduction in the number of MRSA in corneas than with gatifloxacin 0.3% or moxifloxacin 0.5% ($P < 0.01$ for early treatment; $P < 0.001$ for late treatment) [70, 71]. Besifloxacin has also been found more effective ($P < 0.05$) than gatifloxacin and moxifloxacin in reducing

bacterial loads in rabbit corneas infected with a resistant strain of *P. aeruginosa* [72].

Antibacterial Prophylaxis for Surgery

No topical antibiotic is currently approved for prophylactic use in ocular surgery. Given the low rate of postoperative endophthalmitis [73, 74], prospective studies of topical antibiotic prophylaxis would require extremely large study populations. Nevertheless, use of topical antibiotics—particularly fourth-generation FQs—as surgical prophylaxis is considered a standard of care [75, 76]. Retrospective studies suggest that perioperative use of fourth-generation FQs such as moxifloxacin or gatifloxacin is associated with low rates of endophthalmitis after cataract surgery [76, 77].

Human and animal data from previously mentioned pharmacokinetic studies suggests that intraocular penetration of FQs, in general, is minimal [49, 51–53]. Since topical administration of current FQs cannot achieve high aqueous humor drug concentrations, an appropriate goal for topical antibiotics in surgical prophylaxis is the reduction of the number of pathogens on the ocular surface. Indeed, the normal microflora on the eyelids and conjunctival sac is the main source of the bacteria associated with endophthalmitis, the predominant causative organisms being Gram-positive species, most commonly CoNS (*S. epidermidis*) [78, 79].

Bucci et al. [80] randomized 67 cataract surgery patients to receive besifloxacin or moxifloxacin before surgery to compare the FQs' antibacterial efficacy. Patients instilled study drug four times a day for 3 days in the surgical eye and 1 h before surgery in the nonsurgical fellow eye, and bacterial load was assessed at the time of surgery. While both agents reduced the bacterial load on the lid margin when administered four times daily for

3 days ($P \leq 0.019$), only besifloxacin reduced the lid colony counts within 1 h of instilling a single drop to nonsurgical eyes ($P = 0.039$). In vitro susceptibility testing of baseline isolates recovered from lid margins and conjunctiva of these patients shows that besifloxacin had greater activity for CoNS than vancomycin (MIC₉₀: 0.5 vs. 2 µg/mL) and an eightfold lower (better) MIC₉₀ for MRSE than moxifloxacin. These data, taken together with the lack of postoperative infections reported with besifloxacin use in the surgical setting [81–85], suggest that besifloxacin may effectively reduce ocular surface flora prior to or after surgery.

Bacterial Lid Disorders

Besifloxacin ophthalmic suspension 0.6% has been investigated for the treatment of acute blepharitis and congenital nasolacrimal duct obstruction (NLDO) with infection in two small pilot studies. John et al. compared twice daily besifloxacin 0.6% and erythromycin ophthalmic ointment 0.5% in a randomized study of 30 patients with acute symptomatic anterior (with or without posterior) blepharitis [86]. While all patients experienced improved clinical signs ($P < 0.05$ for both groups) and symptoms ($P < 0.005$ for both groups) following two weeks of antibiotic treatment alongside lid hygiene measures, the besifloxacin group showed a greater reduction in bacterial loads. Of the 13 besifloxacin-treated *Staphylococcus* isolates (including 5 multidrug resistant organisms), 6 showed no growth post-treatment and 7 showed limited growth of *S. epidermidis*. In contrast, six of the erythromycin-treated isolates demonstrated increased growth of organisms after treatment.

To compare the use of besifloxacin for the treatment of congenital NLDO with infection with trimethoprim/polymyxin (Polytrim[®],

Allergan, Irvine, CA, USA), Tu et al. [87, 88] randomized 24 children aged 1–12 months with diagnosed NLDO with infection. Dosed three times a day for 10 days, besifloxacin was found to be as effective as trimethoprim/polymyxin in treating the condition, with success rates of 88% (8/9) and 91% (10/11), respectively. Only one patient in each treatment group suffered recurrent infection.

Safety Profile

Topical ocular administration of besifloxacin achieves high ocular surface concentrations with negligible levels in plasma [43], creating the potential for high therapeutic availability and effectiveness with a minimal risk of systemic side effects. Indeed, besifloxacin demonstrated favorable ocular safety and tolerability in clinical trials of conjunctivitis with besifloxacin administered both three times daily for 5 days and twice daily for 3 days (Table 3), with rare nonocular side effects [59, 89]. Most adverse events in these studies were mild or moderate in severity.

In addition, besifloxacin was well-tolerated by the pediatric patients in the besifloxacin clinical trials dosed three times daily for 5 days ($N = 815$), with similarly low incidences of ocular adverse events found in all treatment groups (besifloxacin, moxifloxacin, and vehicle) [61]. The most commonly reported adverse events in besifloxacin-treated eyes from pediatric patients were conjunctivitis (2.9%), bacterial conjunctivitis (2.1%), and eye pain (1.8%); headache, the only nonocular adverse event reported in more than 1% of patients, occurred in 1–2% of patients in each treatment group.

More recently, Malhotra et al. [90] examined the safety of besifloxacin used 3 times daily for 7 days—the FDA-established recommended

dosing regimen—in 514 patients with bacterial conjunctivitis in a randomized, multicenter, vehicle-controlled, parallel-group study. The rates of ocular treatment-emergent adverse events were similar for besifloxacin-treated and vehicle-treated patients (4.9% vs. 6.5%, $P = 0.5362$). Only 1.2% of besifloxacin-treated patients and 2.9% of vehicle-treated patients reported ocular events considered at least possibly related to treatment, and almost all ocular events were mild or moderate and self-limited. No serious nonocular events were reported; of a total of 16 nonocular events (10 in besifloxacin group and 6 in vehicle group), only one event of self-limited dysgeusia in the besifloxacin group was considered definitely related to treatment. The results, overall, indicate that besifloxacin ophthalmic suspension 0.6% is safe when used three times daily for seven days.

Until very recently, there was no human data on the safety of besifloxacin in the treatment of bacterial keratitis. As indicated earlier, Schechter et al. [69] evaluated the safety as well as the efficacy of besifloxacin in the treatment of 142 patients with bacterial keratitis in the retrospective multicenter study. Only one ocular adverse event of mild punctate keratitis was reported, which resolved without scarring or neovascularization.

The safety of besifloxacin for antibacterial prophylaxis in ocular surgery has been studied to a greater extent. A retrospective chart review of 801 laser-assisted in situ keratomileusis (LASIK) cases found that perioperative use of besifloxacin ($n = 534$; 2–4 times daily, mean treatment duration: 8.6 days) and moxifloxacin ophthalmic solution 0.5% ($n = 267$; 4 or more times daily, mean treatment duration: 8.0 days) in patients undergoing LASIK surgery was not associated with any adverse drug reaction [83]. Similarly, a recent prospective, multisite, LASIK

Table 3 Treatment-emergent ocular AEs with $\geq 1\%$ incidence (unless specified otherwise) of study eyes in any treatment group from besifloxacin clinical trials

Variables	Three times daily dosing, 5 days*				Twice daily dosing, 3 days**	
	Besifloxacin (<i>n</i> = 1192)	Vehicle (<i>n</i> = 616)	Moxifloxacin (<i>n</i> = 579)	<i>P</i> value (Fisher's exact test)	Besifloxacin (<i>n</i> = 228)	Vehicle (<i>n</i> = 236)
Total number of AEs	191	146	81	–	12	14
Number of eyes with ≥ 1 AE	139 (11.7)	101 (16.4)	54 (9.3)	0.006	12 (5.3)	12 (5.1)
Blurred vision	25 (2.1)	24 (3.9)	3 (0.5)	0.032	–	–
Eye irritation	17 (1.4)	18 (2.9)	8 (1.4)	0.046	–	–
Eye pain	22 (1.8)	11 (1.8)	7 (1.2)	>0.99	–	–
Conjunctivitis	14 (1.2)	15 (2.4)	5 (0.9)	0.049	5 (2.2)	5 (2.1)
Eye pruritus	13 (1.1)	10 (1.6)	2 (0.3)	0.38	–	–
Conjunctivitis, bacterial	7 (0.6)	9 (1.5)	2 (0.3)	0.068	2 (0.9)	1 (0.4)

Values expressed as *n* (%)

AE adverse event

* Pooled data from six clinical and Phase I safety studies [89]

** No significant difference between treatment groups; all *P* values (Fisher's exact test) were >0.2 [59]

safety surveillance study by Majmudar and Clinch [85] suggested besifloxacin appears safe for surgical prophylaxis; among the 456 study eyes (besifloxacin: *n* = 344; moxifloxacin, *n* = 112), no treatment-emergent adverse events were reported.

However, problems with the prophylactic use of besifloxacin in the surgical setting have been reported under particular circumstances. Talamo et al. [91] reported delayed epithelial closure (5 to 13 days, with an average of 8.8 days) and delayed visual recovery in a case series of 4 patients (7 eyes) treated with besifloxacin 0.6% instilled underneath a bandage contact lens (BCL) placed at the conclusion of photorefractive keratectomy (PRK) [91]. These adverse reactions were attributed by the authors to potential toxic effects of DuraSite or the preservative benzalkonium chloride 0.01% on exposed

corneal stroma, especially when drug contact time is prolonged. Consistent with this premise, a joint alert issued by the American Society of Cataract and Refractive Surgery (ASCRS) Cornea and Refractive Surgery Committees in 2013 recommended withholding topical ophthalmic medications with advanced vehicles immediately prior to or intraoperatively during LASIK or PRK while the stromal bed is exposed [92].

To date, there has been no other evidence in the literature for such adverse events with the besifloxacin formulation. Donnenfeld et al. [84] evaluated the effect of besifloxacin 0.6% or moxifloxacin 0.5% (Vigamox) on epithelial wound healing following PRK in a prospective, contralateral eye, double-masked, multicenter study. A total of 80 eyes (40 patients) were randomized to either besifloxacin or moxifloxacin administered 3 times daily after

the BCL was placed and until the cornea was healed. The two groups demonstrated no difference ($P = 0.763$) in epithelial wound healing, with mean time to complete epithelial closure 80.9 ± 11.8 h (range 3–5 days) for besifloxacin-treated eyes and 82.4 ± 12.1 h (range 3–5 days) for the moxifloxacin-treated eyes. These results are consistent with findings in animal models of corneal epithelial defects that neither DuraSite nor besifloxacin negatively affects corneal reepithelialization [93, 94].

Concerns have also emerged in regards to suture-less clear corneal surgery, where it has been suggested that a leaking wound could give the DuraSite vehicle entry to the anterior chamber, block the trabecular meshwork, and cause significant anterior chamber toxicity [95, 96]. Studies in patients undergoing routine, uncomplicated, suture-less cataract surgery, however, have thus far produced no clinical evidence that prophylactic use of besifloxacin is associated with any significant safety concerns. A randomized, parallel-group, investigator-masked study of 58 patients undergoing suture-less clear cornea surgery reported no adverse events with either besifloxacin or moxifloxacin used prophylactically (both administered 4 times daily starting 3 days prior to surgery and continued for 7 days postoperatively) [81]. Similarly, Parekh et al. [82] found no evidence of adverse drug reactions following besifloxacin or moxifloxacin prophylaxis in a retrospective chart review of more than 700 consecutive cases of routine cataract surgery obtained from nine clinical centers in the US (besifloxacin: $n = 493$, 89% suture-less; moxifloxacin: $n = 253$, 78% suture-less) [82]. Finally, in a prospective, multisite, cataract surgery surveillance study of 485 eyes (besifloxacin: $n = 333$; moxifloxacin: $n = 152$) conducted by Majmudar and Clinch

[85], only 1 treatment-emergent adverse event (mild hypersensitivity or allergic reaction) was reported in a besifloxacin case, and this resolved after discontinuation of medication.

CONCLUSIONS

Besifloxacin is a novel topical C8-chlorofluoroquinolone with potent, broad-spectrum antibacterial activity and a favorable pharmacokinetic profile that together supports its use in the empirical treatment of bacterial infection at the ocular surface. Besifloxacin has been established as an effective and safe treatment for bacterial conjunctivitis, while further investigations are needed to assess its safety and efficacy in bacterial keratitis, antimicrobial prophylaxis in ocular surgery, and for the treatment of bacterial lid disorders. Compared with other topical FQs, besifloxacin ophthalmic suspension offers several potential therapeutic advantages, including higher ocular surface drug concentrations, longer ocular surface exposure times, and greater efficacy against FQ-resistant ocular pathogens, including MRSA and MRSE.

ACKNOWLEDGMENTS

Sponsorship for this article was provided by Bausch + Lomb, a division of Valeant Pharmaceuticals International, Inc., Rochester, NY, USA. All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this manuscript, take responsibility for the integrity of the work as a whole, and have given final approval to the version to be published. Editorial assistance in the preparation of this manuscript was provided by Ethis

Communications Inc. Support for this assistance was funded by Bausch + Lomb. The authors also thank H. H. DeCory (Bausch + Lomb) for critical review of content and editing of the manuscript, and A. Jeganathan (Bausch + Lomb) for assistance with chemical structures. The article processing charges for this publication were funded by Bausch + Lomb.

Disclosures. Francis S. Mah discloses being a consultant for Alcon, Allergan, Bausch + Lomb, Foresight, Ocular Therapeutix, Polyactiva, and Shire. Christine M. Sanfilippo discloses being an employee of Bausch + Lomb.

Compliance with Ethics Guidelines. This article is based on previously conducted studies, and does not involve any new studies of human or animal subjects performed by any of the authors.

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